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STUDY THE PATHOLOGICAL EFFECTS OF CRUDE EXTRACT OF *PORTULACA OLERACEA* L. IN THE TREATMENT OF TRANSPLANTED MAMMARY TUMOR IN FEMALE ALBINO MICE

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ABSTRACT
This study was designed to evaluate the therapeutic effect of 70% ethanolic crude extract of *Portulaca oleracea* L on mice mammary adenocarcinoma (*in vivo). In vivo*, the acute toxicity of 70 % ethanolic extract of the plant on normal mice has been studied. No toxic effect was noticed on normal mice even at 9500 mg /kg B.W S/C injection. Therapeutic effect of ethanolic extract of *Portulaca oleracea* was studied on tumor- bearing female mice after S/C administration at dose of 200 mg/kg B.W for 30days (group II), compared with tumor- bearing female mice (group I) were injected with D.W only and served as control (+). While healthy female mice (group III) injected with D.W only and served as a control (-). The results showed significant reduction in tumor volume, relative tumor volume and inhibition of tumor growth rate in treated mice (group II) compared with tumor-bearing female mice of non-treated group (group I), which showed increased tumor volume. Gross lesion of tumor mass (group II) showed a small size tumor mass and localized S/C region. Histologically, showed extensive tumor cell necrosis in the center, surrounded by a thick band of granulation tissue which is infiltrated with mononuclear cells (lymphocyte and macrophage). Compared with gross lesion of tumor mass (group I) which revealed that large, irregular tumor mass, with highly vascularzation. Histologically, there was extensive tumor growth which consist of aciner like structure, involving C.T stroma of mammary gland, the tumor cells are hyperchromatic, pleomorphic, giant tumor cell formation and certain section showed mitotic figure, some tumor growth showed extensive coagulation necrosis in the center, with area of calcification.

KEY WORDS: Crude Extract of *Portulaca oleracea* L., treatment mice mammary adenocarcinoma

INTRODUCTION

Cancer is one of the dangerous diseases which affect humans and animals. The national agency for cancer research estimated 10.9 million cases yearly, 6.7 million people died and 24.9 million people suffering from cancer [1]. The American Cancer Society stated that cancer is the second leading cause of death in the US, exceeded only by the heart diseases [2]. In Iraq, the total incidence rate in the 2001 reached to 61.83 cases per 100,000 individuals [3]. Several limitations make conventional therapies less effective, for example, secondary tumors that metastasized from the primary foci, and other leukemias make the surgical therapy to be limited and less effective [4]. Plants contain different phytochemicals with biological activities that can provide therapeutic effects that may be useful in healing and reducing the risk of cancer[5]. Natural products have been long been a fertile source of cure for cancer , there are at least 250,000 species of plants out of which more than one thousand plant have been found to posses significant anticancer properties [6] . Natural products play a dominant role in cancer chemotherapeutics with more than 70% of anticancer compounds being either natural products or derived from natural products[7]. These include Vincristin, Vinblastin alkaloid from *Vinca rosea* which have high activity against acute mylocytic leukemia [8]. In Iraq, there are many attempts to study the cytotoxic effect of local plant extract using cancer cell lines like HEP-2, Vero, Hela, Ref, Amn3 and RD cell lines *in vitro*, and AM3 cell line *in vivo.* Such studies include *Withania somnifera* [9], royal jelly and propolis [10], *Urtica dioica* [11].

Portulaca oleracea L (Purslane), has many folkloric uses, it is used in the Arabian peninsula as antiseptic, anti-scorbutic, antispasmodic [12]. In China, it is used as an anti-bacterial and anti-viral agent[13]. *Portulaca oleracea* L showed a tumoricidal activity against KATO III (human gastric carcinoma cell line) and COLO 320 HSR cells (human colon adenoma cell line) *in vivo* and *in vitro* [14]. Purslane acts as analgesic, antiarthritic, antiartheriosclerotic and anticancer (colon, stomach, liver and skin) activities[15]. Also[16] mentioned that Polysaccharide from *Portulaca oleracea* L. has immune effects on mice with tumor S180. This study was designed to assess the possible therapeutic effects of *portulaca oleracea* crude extract through performing the following aim: - Study the pathological effects of ethanolic extract of *portulaca oleracea* L, on growth of transplanting tumor in female mice *in vivo.*

MATERIALS & METHODS

1- Collection and extraction of plant

Portulaca oleracea plant was obtained from field of College of Veterinary Medicine, University of Baghdad. Representative specimens (leaves and stems) were taken to the College of Science, Botany Department, University of

Baghdad and identified by Professor Dr. Ali- AL-Mosawy as *Portulaca oleracea* L, Family Portulacaecea. Plant extraction was done According to [17].

2- Median lethal dose

Graduated doses of *Portulaca oleracea* ethanolic extract were dissolved in 10 ml distill water and administered S/C as 0.1 ml for each 10 gm of animal body weight. The range was of S/C single doses used in the determination of LD50 of the extract was (5000- 9500) mg /kg B.W. Mortality was recorded after 24 hrs and LD50 was calculated according to up and down method described by [18].

3- Animals treated with ethanolic extract of *Portulaca oleracea*

By returning to the results of LD50, and values reported in some references [14], the dose used in this study was (200 mg/ kg B .W after S/C injection daily for 30 days.

4-The effect of extract on tumor growth *in vivo*

Transplantation of tumor cells in mice: Single tumor mammary adenocarcinoma bearing mouse (AM3) was supplied from Iraqi Center for cancer & Medical Genetics Research/ University of AL-Mustanseraia ICCMGR. This mouse was used to obtain tumor cells which later transplanted into adult female albino mice. The following protocol was followed to perform the transplantation process [19].

Treatment of tumor by using plant extracts

Once the tumor reached the suitable volume (at least 5 mm). Eight female adult mice were used at 8-10 wks of age and 25-30 gm B.W, kept in Iraqi Center for cancer & Medical Genetics Research/ University of AL-Mustanseraia, and given pellets of balanced specially prepared animal feed and water *ad libitum.* These animals were divided into two treatment groups (each contains 4 adult female albino balb/C mice).

I- Four adult female albino mice bearing tumor mass injected S/C daily with D.W for 30 days (control positive).

II-Four adult female albino mice bearing tumor mass injected S/C daily with 200 mg / kg B. W of ethanolic extract of P. O for 30 days (treated group).

III. Four healthy adult female albino mice injected S/C daily with D.W for 30 days (control negative).

Tumor volume (TV) (mm3), were observed and recorded by using vernier caliber according to (20).

The inhibition rate of tumor growth (GI %) was calculated according to [21].

The relative tumor volume (RTV): was calculated according to [22].

After complete (30) days for the three groups, the animals were killed by inhalation anesthesia and tumor mass were taken from group I and II. Then fixed in 10%formaline in order to study histopathological changes.

Statistical analysis

Statistical analysis of data was performed by using Statistical Package for Social Science, (SPSS) (2008), Version 16, and for determination of significant differences using [23].

RESULTS & DISCUSSION

Median lethal Dose (LD_{50})

Acute toxicity test of *portulaca oleracea* extract showed no toxic symptoms on the animals when extracted by 70 % ethanolic solution. Different doses ranging from (5000 to 9500) mg/kg B.W injected subcutaneously caused no deaths in experimental mice. *Portulaca oleracea* was considered safe even at high dosage [24].

Effect of ethanolic extract of *Portulaca oleracea* on transplanted mammary tumor in mice

Subcutaneous injection with ethanolic extract of *Portulaca oleracea,* at a dose 200mg /kg B.W for 30 days, showed highly significant decrease($P < 0.01$) in tumor volume in tumor-bearing female mice (group II) especially at the last days of experiment(170.75±10.62 ,154.88±17.41and 141.88±10.75), compared with tumor-bearing female mice which treated with distilled water and served as control(group I) which recorded highly significant increase in tumor volume($P<0.01$) at last days (4508.75±345.95, 6889.13±306.41 and 9539.63±371.45) respectively. Relative tumor volume showed a highly significant decrease ($P<0.01$) in the treated group after 30 days of the experiment (56.51±0.45), compared with non treated group which recorded a highly significant increase ($P< 0.01$) in RTV (3615.27 ±4. 10). Treatment of tumor bearing female mice with 200 mg/kg B.W with *Portulaca oleracea* plant extract revealed a highly significant tumor growth inhibition ($P<0.01$) (98.51±0.82) after 30 days of the experiment. Researchers reported a similarity in characterization between murine mammary adenocarcinoma and human mammary adenocarcinoma [25]. And some of them were used TSA cell line as typical pattern for murine mammary adenocarcinoma, this TSA cell line arose as spontaneous tumor growth in female BALB/c mice aged (20 months) multipartum [26]. Ahmad Majeed 2003 cell line (AM3) resembles TSA cell line from its origin *i.e.* from aged BALB/c multipartum mice [19]. There are three parameters used in the evaluation of tumor growth after S/C injection of ethanolic extract of P.O in mice (group II) compared with control mice (group I). Tumor of non treated group (group I) showed a highly increased tumor size, relative tumor size in a time dependent manner, this indicated that the tumor has had highly aggressive features .While treated group (group II) showed a smaller tumor size, smaller relative tumor volume and less growth inhibition percentage. Ethanolic extract of *Portulaca oleracea* has essential phytochemical compounds such as alkaloids, flavonoids, glycosides, saponines and tannins, were a positive reaction to phytochemicals analysis. These compounds are widely distributed in plant Kingdom, and have cytotoxic and antiproliferative effect against cancer cells [27]. Our interpretation to reduce tumor volume in this study in tumor bearing mice treated with P.O and inhibition tumor growth were by the action these phytochemical compounds against tumor cells.

Other mechanisms have been proposed for suppression of tumor cell growth by omega 3 fatty acids. When omega 3 fatty acids are available in the diet, they will be used as a substrate by cycloxygenase (COX2), it has been reported that DHA fatty acid inhibits eicosanoid synthesis from arachedonic acid (AA) [28]. Ecosapentanoic acid (EPA) is a better substrate for COX than AA, and EPA competes more successfully than AA for COX activity [29]. The result is that if omega 3 fatty acids are included in the diet will less of the inflammation-producing and growth-promoting prostaglandin E2 will be produced in normal and in tumor tissues. The omega 3 fatty acids decrease activation of oncogenic transcription factors *ras* and AP1 [30], which are

transcription factors for many growth-promoting genes. Thus omega 3 fatty acids can slow growth of cancer cells by direct action and by their activity as second messengers [31]. Yoon and colleagues [14], mentioned that P.O has tumoricidal activity against KATO III (Human gastric carcinoma cell line) and COLO 320 HSR (Human colon adenocarcinoma*) in vivo* and *in vitro* and not a normal cell line. *Portulaca oleracea* contain large amount of dopamine and may possibly play a role as antitumor. Dopamine may inhibit the production or release of endogenous factors required for cell viability and proliferation [32], and specifically inhibits the VPF/VEGF – induced angiogenesis by acting on D2 dopamine receptors present on endothelial cells (33).

PATHOLOGICAL STUDY

Pathology of mammary adenocarcinoma - non treated group (Group I)

Gross lesion revealed that large, irregular, numerous numbers of blood vessels with necrotic area (Fig 1). Histologically, there was extensive tumor growth consisted of aciner like structure, involving C.T stroma of mammary gland (Fig 2), the tumor cells are hyperchromatic, pleomorphic, increase the nuclear-cytoplasmic ratio, giant tumor cell formation (Fig 3) and certain section showed an extensive mitotic figure, some tumor cells showed extensive coagulation necrosis in the center, with area of calcification(Fig 4) .

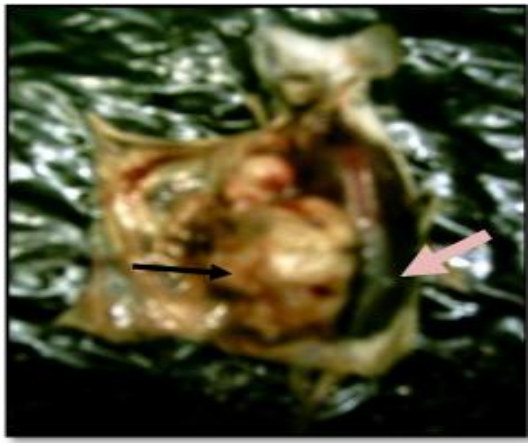

FIGURE 1: Gross lesion of tumor-bearing mice treated with distil water for 30 days as a control (group I) showed large, irregular highly vascularised tumor mass ( ) with large area of necrotic tissue. ( ).

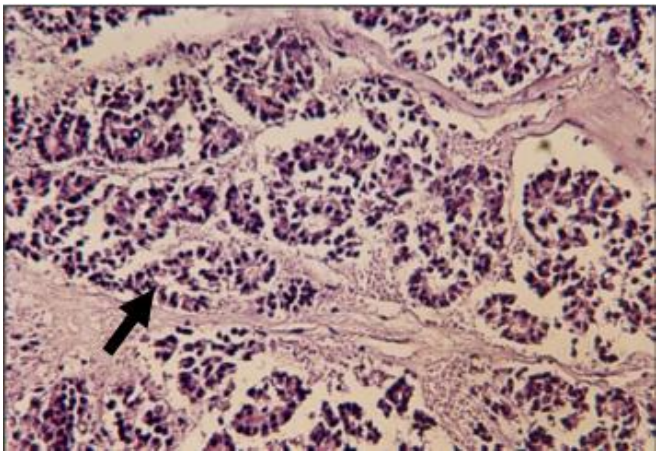

FIGURE 2: Histopathological section of mammary adenocarcinoma in tumor-bearing female mice treated with distilled water for 30 days as a control group (groupI), showing acinar like structure ( ) involving C.T stroma of mammary gland (200XH&E).

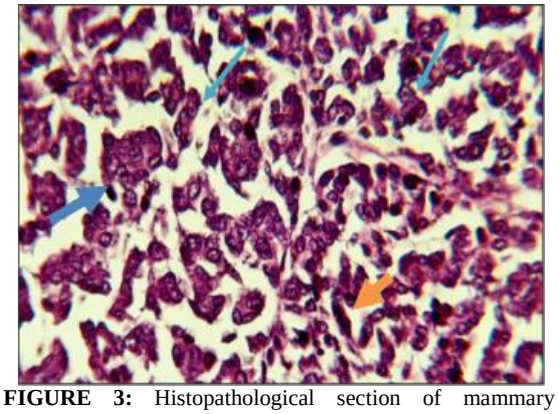

FIGURE 3: Histopathological section of mammary adenocarcinoma in tumor-bearing female mice treated with distilled water for 30 days as a control group (group I), showing pleomorphic tumor cell ( ), hyperchromatic ( ), increased nucleus: cytoplasmic ratio and giant tumor cell formation ( ) (400XH&E).

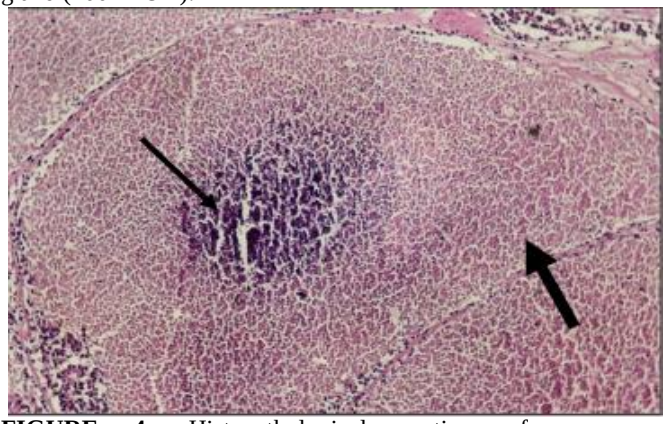

FIGURE 4: Histopathological section of mammary adenocarcinoma in tumor-bearing female mice treated with distilled water for 30 days as a control group (group I), showing central calcification ( ) in the large area of necrosis ( ) (100XH&E). (200XH&E).

Pathology of mammary adenocarcinoma - treated group (Group II)

Grossly tumor mass showed a small size tumor mass and localized S/C region (Fig5). Histologically, showed extensive tumor cell necrosis in the center, surrounded by a thick band of granulation tissue which is infiltrated with mononuclear cells (lymphocyte and macrophage) (Fig6). Histopathological section of tumor growth of the treated and non treated groups was performed to analyze the process of anti tumor .Highly lymphocytic infiltration were observed around the tumor cells in treated group ,these results referred to mice acquired on immunological

memory for tumor cells and induction of tumor – specific cells mediated immunity .There were few tumor cells in treated group and fibrous connective tissue(F.C.T) formation surrounding tumor masses was identical to that described for healing processes by fibrosis i.e. organization of fibrin with formation of granulation tissue which subsequently mature into F.T.C replacing the progressively necrotic tissue [34], and leaded to inhibition of tumor growth ,there was large area of necrotic tumor cells which was surrounding by F.C.T , The necrosis is a characteristic histological feature in treatment which subsequently replaced by fibrous connective tissue as well as marked reduction in the tumor tissue .The interpretations for the growth inhibition effect against transplanted tumor agreed with suggestion of [35]. That was the tumor growth inhibition could be through influence of the natural phenomenon of programmed cell death are likely to be potentially useful drugs. In Contrast, scattered infiltration of lymphocytes could be observed in non treated group, large tumor cells, mitosis of nuclei, progression, invasion, destruction of surrounding area and some tumor cells reached to blood vessels. The hallmark of the malignant tumor is its capacity to spread to, and grow progressively in, tissue remote from its site of origin. Spread may occur by lymphatic vessels or by blood vessels, tumor cells disseminated by the bloodstream may involve any organ, but the lungs, liver and bone marrow are especially common sites of secondary tumor[32], [11]recorded metastasis cases of mammary adenocarcinoma to lung parenchyma. Also other side of tumor growth, there were large area of necrosis with multifocal of dystrophic calcification; this could be interpretation that rapid proliferation of tumor cells may outstrip the capacity of new vessels to supply adequate oxygen and nutrient. The resulting patchy necrosis is characteristic of rapidly growing malignant tumor[36]. While dystrophic calcification is encountered in the area of necrosis of any type and derived from degenerating cells[37].

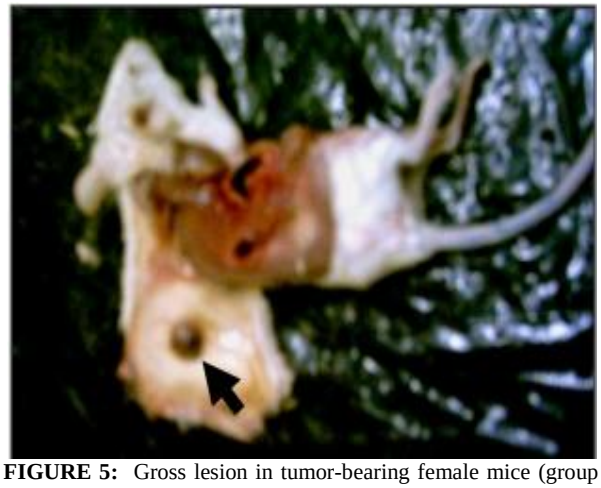

FIGURE 5: Gross lesion in tumor-bearing female mice (group II) treated with 200mg/kg B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days ,showed small tumor size and localized in the S/C region ( ).

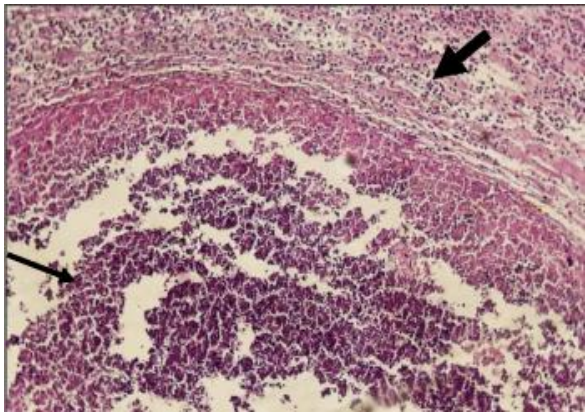

FIGURE 6: Histopathological section of mammary adenocarcinoma in tumor-bearing female mice (group II) treated with 200mg/kg B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days, showed extensive tumor cells necrosis in the centre ( ), surrounded by a thick band of granulation tissue which is infiltrated with mononuclear cells ( )(200X H&E).

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